Kidney International, Vol. 62, Supplement 82 (2002), pp. S73–S80

Cardiovascular calcification in patients with end-stage renal disease: A century-old phenomenon

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Cardiovascular calcification in patients with end-stage renal disease: A century-old phenomenon. The mortality risk from cardiovascular disease is increased in patients with end-stage renal disease (ESRD). This is due to both traditional and dialysis-specific factors. Recently, a number of the dialysis-specific risk factors have been implicated in the pathogenesis of cardiovascular calcification. These include: hyperphosphatemia, high calcium-phosphate (Ca × P) product, elevated parathyroid hormone levels, duration of dialysis, and treatment with calcium-containing phosphate binders and vitamin D analogs. The recent availability of electron beam computed tomography (EBCT) has triggered increased awareness of the occurrence of cardiovascular calcification in ESRD patients. Given the development of transient hypercalcemia with calcium-containing binders, a link between calcium load from use of calcium-containing phosphate binders and development coronary calcification has been proposed. However, a causal relationship between use of these agents and cardiovascular calcification has not been established. Moreover, this phenomenon had been recognized over a century ago, long before these phosphate binders became available. Although its pathogenesis is likely to be multifactorial, available data strongly implicate elevated serum phosphorus as the primary culprit. Furthermore, the risk of calcification may be aggravated by vitamin D therapy, particularly in patients with severe secondary hyperparathyroidism. Therefore, achieving vigorous control of serum phosphorus, Ca × P product and parathyroid hormone level might decrease cardiovascular calcification and improve survival of patients on maintenance hemodialysis. Since calcium acetate is the most cost-effective phosphate binder available, we recommend that it should remain the first line treatment of hyperphosphatemia in patients with ESRD.

The risk of morbidity and mortality in patients with end-stage renal disease (ESRD) is high. Cardiovascular disease is extremely common and accounts for at least 50% of deaths among these patients [1]. Moreover, mortality from cardiovascular disease 20- to 30-fold higher than in age-, gender- and race-matched controls. The burden of cardiovascular disease is even greater in older diabetic patients who now represent the main patient population contributing to the increasing incidence of ESRD in the U.S. There are a number of traditional and dialysis-specific cardiovascular risk factors that may contribute to this burden. Until recently, traditional risk factors such as left ventricular hypertrophy, hypertension, poor glycemic control and dyslipidemia have been targeted as the main causes of the accelerated atherosclerosis and increased mortality in dialysis patients. More recently, a number of dialysis-specific pathogenetic factors also have been implicated including hyperphosphatemia, high Ca × P product, elevated parathyroid hormone levels, as well as treatment with calcium-containing phosphate binders and vitamin D analogs [2].

Coronary artery calcification is absent in normal vessels but usually occurs when atherosclerosis is present. Occasionally it may be detected in early atherosclerotic lesions that develop in the second and third decades of life, but it occurs more frequently in advanced lesions and in older individuals [3]. Electron-beam computed tomography (EBCT) and spiral computed tomography (CT) are highly sensitive techniques for detecting coronary artery calcification and are being used with increasing frequency for screening asymptomatic individuals at risk for developing coronary artery disease [3]. Currently, there is intense interest in the use of these non-invasive techniques for screening patients with ESRD for the presence of occult coronary artery disease. In non-uremic patients, the presence of coronary artery calcification has been shown to predict coronary artery events such as myocardial infarction [4]. There is no doubt that the prevalence of coronary artery calcification, as well as valvular and myocardial calcification is increased in patients with ESRD [5–8]. These abnormalities are usually clinically silent but can be detected by fluoroscopy, conventional computed tomography, spiral CT or EBCT. Quantification of the calcium load in the coronary arteries by EBCT is thought to reflect the total atherosclerotic plaque burden. Therefore, the use of

Key words: hyperphosphatemia, hypercalcemia, phosphate binder, calcium phosphate product, calcium acetate, Sevelamer, hydrochloride, vitamin D, parathyroid hormone, EBCT, osteoblast.

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EBCT offers the potential to evaluate the progression, stabilization, and even regression of calcification through serial imaging. Despite the promise of EBCT as a screening test for coronary artery calcification, the technique has not been standardized with regard to tomographic slice thickness. Furthermore, the reproducibility of the calcium scoring system remains to be established [3, 4].

While the advent of these newer noninvasive screening tests has increased awareness in the nephrology community of the importance of soft tissue and vascular calcification in dialysis patients, interest in this phenomenon is not new. In 1855, Virchow reported five cases of "metastatic calcification" and renal disease was present in three of them [9]. In his comprehensive review of this subject in 1947, Mulligan found 27 autopsy cases of chronic renal disease with metastatic calcification; 15 had vascular calcification involving the aorta, coronary arteries, as well as the renal, mesenteric, hepatic and peripheral arteries [10]. Furthermore, 11 of these patients had myocardial and valvular calcification. Calcium deposits were found both in the intima and media of these arteries. In the early 1970s, the incidence of metastatic calcification in dialysis patients was reported to vary between 38 and 81% [11, 12]. Arterial calcification was even reported among patients with chronic renal failure who were not yet on dialysis. In 1976, Meema, Oreopoulos and deVeber reported that arterial calcification developed in 38% of patients with creatinine clearance less than 20 mL/min who had not been previously dialyzed, 43% in those beginning dialysis, and 44% among dialysis patients [13]. Repeated radiologic studies showed that arterial calcification progressed in 36% and 10% of their non-dialyzed and dialyzed patients, respectively. They speculated that progression of arterial calcification was not due to dialysis *per se*, but to prolongation of life in the presence of chronic renal failure. In an autopsy study of 56 dialyzed and 18 non-dialyzed patients with chronic renal failure, Kuzela and colleagues found calcification in 79% of dialyzed patients and 44% in the non-dialyzed patients [14]. Calcification was severe in 35% of patients and involved the cardiac muscle, conduction system, coronary arteries as well as visceral organs including the lung, stomach and kidneys.

CLINICAL AND PATHOLOGICAL FORMS OF EXTRAOSSEOUS CALCIFICATION

There are three types of extraosseous calcification: calcification in medium-sized arteries, periarticular calcification, and visceral calcification (heart, lung, and kidney). Vascular calcification follows two distinct pathologic patterns [2]. Intimal calcification, which occurs in association with atherosclerotic plaques, is usually a focal process as adjacent areas of the vessel are not involved. Medial calcification of arteries, the so-called Monckeberg's sclerosis, may be found in the absence of atherosclerosis and is characterized by diffuse calcification of the media, particularly at the level of the internal elastic lamina. This form of calcification is commonly seen in the coronary arteries of older individuals, particularly older diabetic patients with ESRD. A subgroup of patients with ESRD may develop a specific form of medial calcification known as calciphylaxis or uremic arteriolopathy. In this type, calcification affects cutaneous and subcutaneous arteries and arterioles, leading to occlusive intimal proliferation. The resulting tissue ischemia leads to painful ulcers on the trunk and extremities and is associated with extremely high mortality.

PATHOGENESIS OF VASCULAR CALCIFICATION

Calcification normally occurs only in bones and teeth. Any calcification outside these organs including blood vessels is considered abnormal. Little is known about the actual mechanisms causing vascular and other tissue calcification. In healthy individuals, abnormal calcification does not occur because of a delicate balance between factors promoting extraosseous calcification and other factors that inhibit calcification [2]. The presence of abnormal calcification implies a disturbance in this balance.

In a series of elegant studies, Alfrey and Ibels concluded that pyrophosphate, an inhibitor of calcium phosphate precipitation in normal urine, plays and important role in abnormal calcification in uremia [15]. Pyrophosphate is normally excreted in the urine but is retained in presence of renal failure. Moreover, high serum phosphorus accelerates pyrophosphate production. Elevated serum levels of pyrophosphate have been reported in uremic patients [16, 17] and bone pyrophosphate is significantly higher in dialyzed patients compared to control subjects [18]. In vitro, pyrophosphate is rapidly adsorbed from the incubation medium onto the surface of apatite crystals [19]. Pyrophosphate is thought to inhibit the transformation of brushite (amorphous calcium-phosphate) to hydroxyapatite (normal bone mineral). These observations are consistent with the finding that the amount of apatite is reduced and the amount of amorphous calcium-phosphate (brushite) is increased in the bones of uremic animals [16]. It has been proposed that accumulation of pyrophosphate in bone may inhibit normal bone mineralization, and paradoxically promote calcium and phosphorus deposition in extraosseous tissues [15]. It is intriguing that in dialysis patients an inverse correlation between coronary artery calcification and bone density has been reported [5]. Moreover, arterial calcification is increased in osteoporotic women and animals with reduced bone mass [20, 21]. It is likely that any pathogenetic process that interferes with bone mineralization, including secondary hyperparathyroidism, adynamic bone disease, and osteoporosis, may predispose to vascular and soft tissue calcification.

The key question is what happens to the calcium and phosphorus that are not deposited into bone in uremic patients? Intuitively, one might think that the calcium and phosphate simply deposit passively in other tissues including blood vessels. Early observations that tissue necrosis is often followed by passive precipitation of calcium-phosphate lead to the traditional view that vascular calcification is a degenerative process that occurs in association with atherosclerosis and normal aging. However, it is remarkable that, almost 150 years ago, Virchow believed that vascular calcification is indeed an active process when he noted that, "*Vascular calcification is an ossification, and not a mere calcification. We see ossification declares itself in precisely the same manner as when an osteophyte forms on the surface of bone*" [22]. In the last decade, the widely accepted notion that extraosseous calcification is a passive phenomenon has once again been seriously challenged, based on strong evidence from experimental and genetic studies suggesting that vascular calcification is an active and highly regulated process similar to bone formation. In this regard, ectopic bone elements, which may even contain bone marrow, have been described in calcified blood vessels, advanced atherosclerotic lesions, and in calcified cardiac valves [23, 24]. These findings suggest that vascular cells have the capacity to transform into osteoblast-like cells capable of producing ectopic bone in the vessel wall [25, 26]. These cells, now called calcifying vascular cells, have been shown to resemble osteoblasts in that they express bone matrix and morphogenic proteins thought to be involved in regulating normal osteogenesis including osteopontin, osteonectin, matrix GLA protein, osteocalcin, bone sialoprotein, and bone morphogenic protein 2a [27]. In particular, expression of osteocalcin, which is pathognomonic of osteoblastic terminal differentiation, strongly suggests that vascular cells assumed an osteoblastic phenotype.

Our understanding of the relevance of these proteins in vascular calcification has been enhanced by the development of knockout models. Mice lacking matrix GLA protein or osteoprotegerin develop medial calcification of arteries [28, 29]. These proteins are expressed in vascular smooth muscle cells where they act as natural inhibitors of vascular calcification. Furthermore, mice lacking Smad6, an inhibitory molecule in the transforming growth factor-β (TGF-β) signaling pathway, have been shown to develop ectopic bone formation in the media of the outflow tracts of the heart [30].

ROLE OF VASCULAR SMOOTH MUSCLE CELLS

The origin of the calcifying vascular cells is not clearly established, but they could be derived from microvascular pericytes or vascular smooth muscle cells (VSMCs) [2]. Pericytes, along with smooth muscle cells and osteoblasts are derived from a common bone marrow mesenchymal stem cell. Both pericytes and smooth muscle cells retain their pluripotent character and therefore have the ability to differentiate into osteoblasts [31]. The osteoblast produces most of the proteins present in bone extracellular matrix (ECM) and it controls the mineralization of this ECM. It is now clearly established that some VSMCs can adopt a calcifying phenotype in vitro and become the so-called calcifying vascular cells. Interestingly, these cells have been shown to resemble osteoblasts and express the same sequence of bone-related proteins (osteopontin, alkaline phosphatase and osteocalcin) expressed by osteoblasts in normal mineralizing bone [32]. Osteocalcin, a protein required for mineralization of ECM, is the only gene expressed in osteoblasts but in no other ECM-producing cell type [33]. Thus, osteocalcin expression by calcifying vascular cells indicates that they have acquired an osteoblastic phenotype. Moreover, these calcifying vascular cells also express the transcription factor cbfa1/osf2, which is absolutely essential for differentiation of mature osteoblasts in bone [34].

ROLE OF CALCIUM IN VASCULAR CALCIFICATION

Intuitively, calcium, phosphorus and the calcium-phosphorus product (Ca × P) should control the precipitation of calcium phosphate at all sites of mineralization, whether in bone, teeth, blood vessels, heart valves or periarticular tissues. However, it is not entirely clear whether these factors, either alone or in combination are primarily responsible for vascular calcification in uremic patients.

The potential role of calcium loading resulting from the use of calcium-containing phosphate binders has recently become an area of major controversy. In a small observational study, Goodman and colleagues reported the presence of coronary calcification in 14 of 16 young adult hemodialysis patients between 20 and 30 years old [6]. They found that patients with coronary calcification were older, had been on hemodialysis for a longer period of time, had higher mean serum phosphorus levels and Ca × P products as well as a greater prescribed daily dose of calcium-containing phosphorus binders. A second small observational study showed that carotid artery compliance and vascular calcification in patients with ESRD were positively correlated with the prescribed daily dose of calcium carbonate [7]. A multicenter trial using EBCT is currently underway that is designed to evaluate progression of coronary artery calcification in dialysis patients treated with calcium-containing binders compared to patients taking sevelamer hydrochloride [8].

Implicit in these reports is the concept that vascular calcification is a passive degenerative process whereby excess calcium simply deposits in extraosseous tissues including blood vessels. This simplistic view ignores the

vast body of evidence outlined earlier that clearly indicates that vascular calcification is an active, highly regulated process. In this regard, several lines of evidence strongly argue against a primary role for calcium loading *per se* in the pathogenesis of vascular calcification.

It should be noted that cardiac calcification in dialysis patients was reported decades before calcium-containing phosphate binders came into widespread use [14]. Furthermore, most clinical studies have failed to demonstrate a significant correlation between the serum calcium level and mortality risk in dialysis patients [5, 35, 36]. In one of the first observational cohort studies on cardiovascular calcification, Braun and colleagues reported an increased incidence of coronary artery, mitral, and aortic valve calcification in hemodialysis patients compared to non-uremic patients with suspected or documented coronary artery disease [5]. They found that age and hypertension independently correlated with the total coronary calcification score. However, no correlation was found with serum calcium levels. Interestingly, there was an inverse correlation between calcification score and bone mass. This observation is in accord with the hypothesis that impaired bone mineralization may be a prerequisite for the development of cardiovascular calcification [21].

The association between coronary artery calcification and binder dose in the above-mentioned observational studies has been employed as the sole basis for targeting calcium-containing binders as the primary cause of increased cardiovascular calcification in dialysis patients. We believe that the paucity of scientific evidence does not justify this conclusion. Results from observational studies should lead to hypothesis testing but cannot be used to establish causation. Moreover, the exclusive emphasis on the potential role of calcium loading in vascular calcification totally ignores the pathogenic role of other important factors such as the adequacy of phosphorus control, vitamin D usage, and parathyroid hormone. Although we cannot exclude a possible role for calcium loading, we believe that vascular calcification is not simply the result of intestinal absorption of excess calcium from calcium-containing binders. Furthermore, the actual calcium load resulting from ingestion of calcium-containing phosphate binders may have been exaggerated. In the absence of vitamin D therapy, intestinal calcium absorption is significantly decreased in patients with ESRD, even when large doses of calcium-containing binders are ingested. Most of this calcium becomes bound to dietary phosphate and both are excreted in the stool. Moreover, reduced levels of 1,25-dihydroxy vitamin D further impair intestinal calcium absorption. Another potential source of calcium loading is the dialysate calcium. However, while the use of a dialysate calcium concentration of 3.5 mEq/L results in a net positive calcium flux, the currently recommended dialysate calcium concentration of 2.5 mEq/L leads to no net calcium flux during dialysis [37]. Since there is no net calcium transfer with the use of low calcium dialysis, and given the impaired intestinal calcium absorption in uremia, one would predict that withdrawal of calcium-containing binders might lead to negative calcium balance. Thus, it is not entirely unexpected that over 50% of patients treated with the non-calcium phosphate binder sevelamer hydrochloride and 2.5 mEq/L dialysate calcium develop hypocalcemia [38]. Accordingly, in order to avoid hypocalcemia, it has been recommended that patients treated with sevelamer hydrochloride receive supplementation with nearly 1 gram of elemental calcium per day [39]. Interestingly, this calcium dose is almost equivalent to the amount of elemental calcium contained in the average dose of calcium acetate required for control of hyperphosphatemia in the Calcium Acetate Renagel Evaluation (CARE) Study as well as other studies [38, 40]. In sevelamer-treated patients, the supplemental dose of calcium carbonate is usually given at night on an empty stomach. Under such conditions, as much as 40% of the administered dose of calcium may be absorbed [41]. In contrast, about 15 to 20% of the elemental calcium is absorbed when calcium acetate is taken with meals [42]. The paradox is that patients taking sevelamer along with supplemental calcium carbonate are likely to absorb just as much calcium as patients receiving calcium acetate with meals as a phosphate binder.

ROLE OF PHOSPHORUS IN VASCULAR CALCIFICATION

Hyperphosphatemia is universally present in patients with ESRD and plays a key role in the development of secondary hyperparathyroidism, extraosseous calcification and calciphylaxis. Moreover, Block and colleagues reported that patients with serum phosphorus levels exceeding 6.5 mg/dL have a 27% increased mortality risk as compared to those with levels between 2.5 and 6.5 mg/dL [35]. Similarly, they found that patients with Ca $\times$ P product exceeding 72 mg^2/dL^2 have a 34% increased risk of death as compared to those with products between 42 and 52 mg^2/dL^2. Since they found no correlation between mortality risk and serum calcium levels, these authors concluded that the risk associated with increased Ca $\times$ P product was driven primarily by phosphorus. Levin and colleagues reported that the increased risk of death was primarily due to cardiac disorders, in particularly coronary artery disease (abstract; Levin et al, *J Am Soc Nephrol* 9:217A, 1998). Similarly, Ganesh et al reported a strong relationship between phosphorus level, Ca $\times$ P product, parathyroid hormone (PTH) level and cardiovascular causes of death, including sudden death [36]. They proposed that serum phosphorus levels exceeding 6.5 mg/dL may be a major cardiotoxin for hemodialysis

patients, and may play a critical role in the development, progression, or rupture of atheromatous coronary artery plaques.

Contrary to the lack of evidence against calcium loading, there are a number of observations that link serum phosphorus levels with vascular calcification. Vascular calcification was clearly documented in predialysis patients with chronic renal failure decades before the introduction of calcium-containing phosphate binders [13, 14]. In that era, hyperphosphatemia also was thought to be the major risk factor for vascular calcification [15].

The molecular mechanisms regulating vascular calcification remain unknown. In an in vitro model, high phosphorus levels in the incubation medium directly enhanced extracellular calcification in human aortic smooth muscle cells via a sodium-dependent phosphate transporter-sensitive mechanism [43]. Moreover, high phosphorus levels increased the expression of the osteoblast-specific genes *Osf2*, *Cbfa*, and osteocalcin. These effects were mediated by up-regulation of the sodium-phosphate cotransporter Pit-1. In the KLOTHO-gene mutant mouse, a twofold increase in serum phosphorus level led to an increased calcium-phosphate product and extensive vascular calcification despite normal renal function [44].

These clinical and experimental observations strongly argue for a primary role for elevated serum phosphorus in the development of vascular calcification in patients with chronic renal failure. It is likely that more vigorous control of serum phosphorus, Ca × P product and PTH might lead to improved survival of patients on maintenance hemodialysis [35, 36]. Consequently, Block and Port recently recommended new goals for serum phosphorus level (2.5 to 5.5 mg/dL) and Ca × P product (<55 mg^2/dL^2) [45]. Although dietary restriction of phosphate is an important first step in effective phosphorus control, when used alone it is often inadequate since phosphorus is present in almost all food products, particularly those with high protein content [46]. Furthermore, thrice weekly hemodialysis alone is not particularly effective in controlling serum phosphorus because most of the total body phosphorus is located in the intracellular compartment [47]. Consequently, phosphate binders are routinely prescribed to patients with end-stage renal disease in order to minimize the intestinal absorption of dietary phosphate. In the last three decades, several phosphate binders have been introduced into clinical practice. Although highly effective, aluminum-containing binders are no longer routinely prescribed because of the toxicities associated with aluminum accumulation [48]. Calcium-containing binders such as calcium carbonate and calcium acetate, and most recently, the non-aluminum, non-calcium, phosphate binder sevelamer hydrochloride now has become the mainstay of treatment for hyperphosphatemia in dialysis patients.

Table 1. Dialysis patients with and without aortic and mitral valve calcification

Risk factors	Valvular calcification $N = 30$	No valvular calcification $N = 80$
Age *years*	64 ± 7[a]	47 ± 8
% with Diabetes	55%[a]	25%
Duration of dialysis *years*	12 ± 4[a]	7 ± 3
Serum calcium *mg/dL*	11.3 ± 2[a]	9.4 ± 3
Serum phosphorus *mg/dL*	6.9 ± 2[a]	5.3 ± 1
Ca × P product mg^2/dL^2	78 ± 9	49 ± 7
PTH level *pg/mL*	735 ± 60	475 ± 83
% with Vitamin D use	90%	10%

[a] $P < 0.01$

ROLE OF VITAMIN D

Administration of vitamin D to ESRD patients with hyperparathyroidism is common practice. Unfortunately, vitamin D substantially increases the intestinal absorption of calcium and phosphorus, and therefore it may increase the Ca × P product and predispose to extraosseous calcification. Furthermore, vitamin D has other effects that may predispose to vascular calcification. Rats treated with high-dose vitamin D developed calcification in the aorta, and the carotid, hepatic, mesenteric, renal and femoral arteries [49]. In other animal studies, prior sensitization with parathyroid hormone or vitamin D resulted in calcification when the animals were subsequently exposed to nonspecific stimuli including trauma [50]. Mallick and Berlyne reported an association between vascular calcification and use of vitamin D therapy in hyperphosphatemic patients with renal failure [51].

Vascular smooth muscle cells have been shown to express vitamin D receptors [52]. Although vitamin D inhibits VSMC proliferation by enhancing calcium flux into cells, it induces VSMCs to exhibit an osteoblastic phenotype [2]. Vitamin D also has been shown to increase calcification in VSMCs in vitro [52–54]. Moreover, vitamin D_3 has been shown to increase the expression of alkaline phosphatase in VSMCs [25]. In a study by one of us, echocardiography in dialysis patients identified 30 patients with valvular calcification and 80 patients without calcification [55]. Several factors were found to be more common in patients with aortic and mitral valve calcification than in those without calcification, including: older age, diabetes, years on dialysis, hypercalcemia, hyperphosphatemia, high Ca × P product, and higher PTH level (Table 1). Of interest, 90% of patients with valvular calcification were receiving vitamin D treatment compared to only 10% of patients without calcification. Thus, both clinical and experimental studies suggest that treatment of dialysis patients with vitamin D could be a major contributing factor to the increased risk of cardiovascular calcification.

ROLE OF PARATHYROID HORMONE

Parathyroid hormone levels have been shown to correlate with increased risk of death in dialysis patients. Ganesh and colleagues found that a PTH level exceeding 495 pg/mL was independently associated with sudden death among their hemodialysis patients [36]. PTH enhances the release of calcium and phosphorus from bone and may increase cytosolic calcium [20].

In addition, PTH-related peptide (PTHrP) has been shown to stimulate VSMCs and osteoblast proliferation, and promote the synthesis of the extracellular matrix required for subsequent calcification [2]. There is a high degree of sequence homology between PTHrP and PTH, and they bind the PTH1R receptor with equal affinity. Many studies have implicated PTH as a permissive factor for cardiac fibroblast activation and myocardial fibrosis [56]. Moreover, animal models have shown regression of myocardial fibrosis after parathyroidectomy [56]. Although available data do not establish causation, it is possible that PTH itself is a cardiotoxin and that markedly elevated PTH levels could increase mortality risk by inducing cardiovascular calcification.

ROLE OF OTHER FACTORS IN VASCULAR CALCIFICATION

The role of inflammatory cytokines in vascular calcification has not been carefully studied. Wang and colleagues reported an increased risk of valvular calcification in peritoneal dialysis patients with elevated C-reactive protein (CRP) and fibrinogen levels, both markers of inflammation [57]. Furthermore, there was a strong association between the degree of hypoalbuminemia and valvular calcification [57]. Chronic renal failure is associated with an increase in serum levels of leptin, which is a satiety factor that regulates food intake and energy expenditure [58]. Leptin has been shown to regulate osteoblastic differentiation and calcification of VSMCs, which are known to express leptin receptors [59]. These observations may explain the association between malnutrition and increased cardiovascular calcification in dialysis patients.

The role of advanced glycation end-products (AGE) in vascular calcification was investigated by Yamagishi and colleagues, who reported that AGE significantly increased the number of calcified nodules in cultured bovine pericytes [60]. They also showed that AGE increased the pericyte expression of alkaline phosphatase and osteopontin mRNAs, markers of osteoblast differentiation.

Finally, warfarin may play a role in vascular calcification. The target for warfarin is matrix Gla protein (MGP), a vitamin K-dependent protein secreted by VSMCs that inhibits vascular calcification [61]. Interestingly, warfarin also has been shown to be a risk factor for development of calciphylaxis [62]. Thus, it is possible that the use of warfarin to maintain vascular access patency may promote the development of vascular calcification.

Table 2. Proposed dialysis-specific risk factors for cardiovascular calcification in uremic patients

Hyperphosphatemia[a] *serum phosphorus >5.5 mg/dL*
Elevated Ca × P product[a] *>55 mg²/dL²*
Exogenous vitamin D therapy[a]
Elevated parathyroid hormone levels *>500 pg/mL*
Treatment with warfarin
Hypercalcemia
Chronic inflammation *elevated C-reactive protein*
Elevated leptin levels

[a] Strong clinical and/or experimental evidence for importance as a risk factor

FUTURE CONSIDERATIONS

From the preceding discussion, it is clear that the issue of vascular calcification in patients with renal failure is more complicated than previously recognized. Although its pathogenesis is likely to be multifactorial (Table 2), we believe that currently available data strongly implicate elevated serum phosphorus as the main culprit in vascular calcification. Therefore, vigorous control of serum phosphorus must remain our primary treatment objective. In the U.S., the two most commonly prescribed phosphate binders are calcium acetate and sevelamer hydrochloride. Previous studies comparing these phosphate binders suggested that they are equally effective in controlling serum phosphorus [63–66]. Unfortunately, these studies used open-label or single arm titration designs. Moreover, in most of these studies, sevelamer hydrochloride was not usually effective in controlling serum phosphorus to the recommended goal of 5.5 mg/dL or less [63, 65, 66]. The recently completed CARE study, a randomized double-blind trial, demonstrated that calcium acetate is significantly more effective than sevelamer hydrochloride in controlling serum phosphorus to the goal level [38]. Calcium acetate-treated patients also had a consistently lower Ca × P product compared to sevelamer hydrochloride-treated patients. Thus, it is conceivable that calcium acetate might reduce the risk of vascular calcification and mortality in dialysis patients as a result of better control of both serum phosphorus and Ca × P product despite somewhat higher, albeit usually normal, serum calcium levels.

Despite the documented efficacy of calcium acetate in treatment of hyperphosphatemia, some investigators have raised concerns about the potential risk of hypercalcemia and cardiovascular calcification with calcium-containing binders [6–8]. Since calcium acetate is twice as effective as calcium carbonate in binding dietary phosphate and contains half the amount of elemental calcium [67], these two drugs should not be used interchangeably

in phosphate binder studies, particularly when evaluating the incidence and long-term consequences of hypercalcemia. Although transient hypercalcemia occurs in dialysis patients treated with calcium-containing phosphate binders, the results of the CARE study demonstrate that in calcium acetate-treated patients, hypercalcemia occurs exclusively in patients receiving concomitant vitamin D therapy [38]. We therefore believe that hypercalcemia can be avoided in the vast majority of calcium acetate-treated patients with the use of low calcium dialysate (2.5 mEq/L) and judicious use of vitamin D. Since the issue of vascular calcification is critical to the very survival of our patients, it must be more carefully evaluated before we accept the overly simplistic notion that vascular calcification is primarily caused by excess calcium intake in the form of calcium-containing phosphate binders. Furthermore, the potential role of vitamin D in vascular calcification has largely been ignored and demands more carefully scrutiny. In our view, the important issue of risk factors for cardiovascular calcification in dialysis patients can only be addressed by well-designed, double-blind studies that control for not only the type of phosphate binder used, but also for the myriad of risk factors potentially associated with vascular calcification including dialysate calcium level, vitamin D and warfarin usage, as well as hyperlipidemia.

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REFERENCES

1. Renal Data System: Causes of death, in *USRDS 1998 Annual Data Report*, Bethesda, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, 1999, pp 63–78
2. Davies MR, Hruska KA: Pathophysiological mechanisms of vascular calcification in end-stage renal disease. *Kidney Int* 60:472–479, 2001
3. Wexler L, Brundage B, Crouse J, *et al*: Coronary artery calcification: Pathophysiology, epidemiology, imaging methods, and clinical implications. *Circulation* 94:1175–1192, 1996
4. O'Rourke RA, Brundage BH, Froelicher VF, *et al*: American College of Cardiology/American Heart Association expert consensus document on electron-beam computed tomography for the diagnosis and prognosis of coronary artery disease. *Circulation* 102:126–140, 2000
5. Braun J, Oldendorf M, Moshage W, *et al*: Electron beam computed tomography in the evaluation of cardiac calcification in chronic dialysis patients. *Am J Kidney Dis* 27:394–401, 1997
6. Goodman WG, Goldin J, Kuizon BD, *et al*: Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med* 342:1478–1483, 2000
7. Guérin AP, London GM, Marchais SJ, Metivier F: Arterial stiffening and vascular calcifications in end-stage renal disease. *Nephrol Dial Transplant* 15:1014–1021, 2000
8. Genzyme (Nasdaq:GENZ): Press release, June 26, 2001. Genzyme study demonstrates significant impact of Renagel on cardiac calcification. www.genzyme.com/ir/genz/press/20010626.html
9. Virchow R: Kall metastasen. *Virchows Arch Pathol Anat* 8:103–113, 1855
10. Mulligan RM: Metastatic calcification. *Arch Pathol* 43:177–230, 1947
11. Parfitt AM, Massry SG, Winfield AC, *et al*: Disordered calcium and phosphorus metabolism during maintenance hemodialysis. Correlation of clinical, roentgenographic, and biochemical changes. *Am J Med* 51:319–330, 1971
12. Conger JD, Hammond WS, Alfrey AC, *et al*: Pulmonary calcification in chronic dialysis patients. Clinical and pathologic studies. *Ann Intern Med* 83:330–336, 1975
13. Meema HE, Oreopoulos DG, deVeber GA: Arterial calcifications in severe chronic renal disease and their relationship to dialysis treatment, renal transplant, and parathyroidectomy. *Radiology* 121:315–321, 1976
14. Kuzela DC, Huffer WE, Conger JD, *et al*: Soft tissue calcification in chronic dialysis patients. *Am J Pathol* 86:403–424, 1977
15. Alfrey AC, Ibels LS: Role of phosphate and pyrophosphate in soft tissue calcification. *Adv Exp Med Biol* 103:187–193, 1978
16. Russell JE, Avioli LV: Effect of experimental chronic renal insufficiency on bone mineral and collagen maturation. *J Clin Invest* 51:3072–3079, 1972
17. David DS, Sakai S, Granda J, *et al*: Role of pyrophosphate in renal osteodystrophy. *Trans Am Soc Artif Intern Organs* 19:440–445, 1973
18. Alfrey AC, Solomons CC, Circillo J, Miller N: Bone pyrophosphate in uremia and its association with extraosseous calcification. *J Clin Invest* 57:700–705, 1976
19. Alfrey AC, Solomons CC, Ciricillo J, Miller N: Extraosseous calcification. Evidence for abnormal pyrophosphate metabolism in uremia. *J Clin Invest* 57:692–699, 1976
20. Cozzolino M, Dusso AS, Slatopolsky E: Role of calcium phosphate product and bone-associated proteins on vascular calcification in renal failure. *J Am Soc Nephrol* 12:2511–2516, 2001
21. Price PA, Faus SA, Williamson MK: The bisphosphonates alendronate and ibandronate inhibit artery calcification at doses comparable to those which inhibit bone resorption. *Arteriscler Thromb Vasc Biol* 21:817–824, 2001
22. Virchow R: *Cellular Pathology: As Based upon Physiological and Pathological Histology.* Dover, New York, 1863 (unabridged reprinting 1971), pp 404–408
23. Bunting CH: The formation of true bone in sclerotic arteries. *J Exp Med* 8:365–376, 1906
24. Parhami F, Bostrom K, Watson K, Demer LL: Role of molecular regulation in vascular calcification. *J Atheroscl Thromb* 3:90–94, 1996
25. Bostrom K, Watson KE, Stanford WP, Demer LL: Atherosclerotic calcification: Relation to developmental osteogenesis. *Am J Cardiol* 75:88B–91B, 1995
26. Jakoby MG 4th, Semenkovich CF: The role of osteoprogenitors in vascular calcification. *Curr Opin Nephrol Hypertens* 9:11–15, 2000
27. Giachelli CM: Ectopic calcification: Gathering hard facts about soft tissue mineralization. *Am J Pathol* 154:671–675, 1999
28. Lou G, Ducy P, McKee MD, *et al*: Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. *Nature* 386:78–81, 1997
29. Bucay N, Sarosi I, Dunstan CR, *et al*: Osteoprotegerin-deficient mice develop early onset osteroporosis and arterial calcification. *Genes Dev* 12:1260–1268, 1998
30. Galvin KM, Donovan MJ, Lynch CA, *et al*: A role for smad6 in development and homeostasis of the cardiovascular system. *Nat Genet* 24:171–174, 2000
31. Shanahan CM, Proudfoot D, Tyson KL, *et al*: Expression of mineralization-regulating proteins in association with human vascular calcification. *Z Kardiol* 89(Suppl 2):II/63–II/68, 2000
32. Stein GS, Lian JB, Stein JL, *et al*: Transcriptional control of osteoblast growth and differentiation. *Physiol Rev* 76:593–617, 1996
33. Ducy P, Zhang R, Geoffroy V, *et al*: Osf2/Cbfa1: A transcriptional activator of osteoblast differentiation. *Cell* 89:747–754, 1997
34. Komori T, Yagi H, Nomura S, *et al*: Targeted disruption of cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell* 89:755–764, 1997
35. Block GA, Hulbert-Shearon TE, Levin NW, Port FK: Association of serum phosphorus and calcium x phosphate product with mortality risk in chronic hemodialysis patients: A national study. *Am J Kidney Dis* 31:607–617, 1998
36. Ganesh SK, Stack AG, Levin NW, *et al*: Association of elevated

serum PO_4, Ca X PO_4 product and parathyroid hormone with cardiac mortality risk in chronic hemodialysis patients. *J Am Soc Nephrol* 12:2131–2138, 2001
37. Hou SH, Zhao J, Ellman CF, *et al*: Calcium and phosphorus fluxes during hemodialysis with low calcium dialysate. *Am J Kidney Dis* 28:217–224, 1991
38. The Podium Report: Hyperphosphatemia, not calcium, is critical link in arterial calcification in dialyzed patients. Presented at the National Kidney Foundation meeting, Chicago, April 19, 2002, pp 1–3
39. Chertow GM, Dillon M, Burke SK, *et al*: A randomized trial of sevelamer hydrochloride (RenaGel) with and without supplemental calcium. Strategies for the control of hyperphosphatemia and hyperparathyroidism in hemodialysis patients. *Clin Nephrol* 51:18–26, 1999
40. Emmett M, Sirmon MD, Kirkpatrick WG, *et al*: Calcium acetate control of serum phosphorus in hemodialysis patients. *Am J Kidney Dis* 17:544–550, 1991
41. Sheikh MS, Santa Ana CA, Nicar MJ, *et al*: Gastrointestinal absorption of calcium from milk and calcium salts. *N Engl J Med* 317:532–536, 1987
42. Sheikh MS, Maguire JA, Emmett M, *et al*: Reduction of dietary phosphorus absorption by phosphorus binders. A theoretical, in vitro, and in vivo study. *J Clin Invest* 83:66–73, 1989
43. Giachelli CM, Jono S, Shioi A, *et al*: Vascular calcification and inorganic phosphate. *Am J Kidney Dis* 38(Suppl 1):S34–S37, 2001
44. Kuro-o M, Matsumura Y, Aizawa H, *et al*: Mutation of the mouse Klotho gene leads to a syndrome resembling ageing. *Nature* 390: 45–51, 1997
45. Block GA, Port FK: Re-evaluation of risks associated with hyperphosphatemia and hyperparathyroidism in dialysis patients: Recommendations for a change in management. *Am J Kidney Dis* 35: 1226–1237, 2000
46. Kopple JD, Coburn JW: Metabolic studies of low protein diets in uremia. II. Calcium, phosphorus and magnesium. *Medicine (Baltimore)* 52:597–607, 1973
47. Mucsi I, Hercz G, Uldall R, *et al*: Control of serum phosphate without any phosphate binders in patients treated with nocturnal hemodialysis. *Kidney Int* 53:1399–1404, 1998
48. Alfrey AC, LeGendre GR, Kaehny WD: The dialysis encephalopathy syndrome. Possible aluminum intoxication. *N Engl J Med* 294:184–188, 1976
49. Price PA, Buckley JR, Williamson MK: The amino bisphosphonate ibandronate prevents vitamin D toxicity and inhibits vitamin D-induced calcification of arteries, cartilage, lungs, and kidneys in rats. *J Nutr* 131:2910–2915, 2001
50. Selye H, Gabbiani G, Strebel R: Sensitization to calciphylaxis by endogenous parathyroid hormone. *Endocrinology* 71:554–558, 1962
51. Mallick NP, Berlyne GM. Arterial calcification after vitamin-D therapy in hyperphosphatemic renal failure. *Lancet* 2:1316–1320, 1968
52. Merke J, Hofmann W, Goldschmidt D, Ritz E: Demonstration of 1,25 $(OH)_2$ vitamin D_3 receptors and actions in vascular smooth muscle cells in vitro. *Calcif Tissue Int* 41:112–114, 1987
53. Neiderhoffer N, Bobryshev YV, Lartaud-Idjouadiene I, *et al*: Aortic calcification produced by vitamin D_3 plus nicotine. *J Vasc Res* 34:386–398, 1997
54. Jono S, Nishizawa Y, Shioi A, Morii H: 1,25-dihydroxyvitamin D_3 increases in vitro vascular calcification by modulating secretion of endogenous parathyroid hormone-related peptide. *Circulation* 98:1302–1306, 1998
55. Ayus JC, Lorenzoy V, Hernandez D: Cardiac complications of renal failure and transplantation (chapt 8), in *Insuficiencia Renal Cronica, Dialysis y Transplante Renal* (2nd ed), edited by Llach P, Valderrábano F, Madrid, Ediciones Norma, 1997, pp 173–203
56. Amann K, Ritz E: Cardiac disease in chronic uremia: Pathophysiology. *Adv Renal Replace Ther* 4:212–224, 1997
57. Wang AYM, Woo J, Wang M, *et al*: Association of inflammation and malnutrition with cardiac valve calcification in continuous ambulatory peritoneal dialysis patients. *J Am Soc Nephrol* 12:1927–1936, 2001
58. Fontán M, Rodríguez-Carmona A, Cordido F, Garcia-Buela J: Hyperleptinemia in uremic patients undergoing conservative management, peritoneal dialysis, and hemodialysis: A comparative analysis. *Am J Kidney Dis* 34:824–831, 1999
59. Parhami F, Tintut Y, Ballard A, *et al*: Leptin enhances the calcification of vascular cells. Artery wall as a target of leptin. *Circ Res* 88:954–960, 2001
60. Yamagishi S, Fujimori H, Yonekura H, *et al*: Advanced glycation endproducts accelerate calcification in microvascular pericytes. *Biochem Biophys Res Comm* 258:353–357, 1999
61. Price PA, Faus SA, Williamson MK: Warfarin-induced artery calcification is accelerated by growth and vitamin D. *Arterioscler Thromb Vasc Biol* 20:317–327, 2000
62. Coates T, Kirkland GS, Dymock RB, *et al*: Cutaneous necrosis from calcific uremic arteriolopathy. *Am J Kidney Dis* 32:384–391, 1998
63. Bleyer AJ, Burke SK, Dillon M, *et al*: A comparison of the calcium-free phosphate binder sevelamer hydrochloride with calcium acetate in the treatment of hyperphosphatemia in hemodialysis patients. *Am J Kidney Dis* 33:694–701, 1999
64. Chertow GM, Burke SK, Lazarus JM, *et al*: Poly[allylamine hydrochloride] (RenaGel): A noncalcemic phosphate binder for the treatment of hyperphosphatemia in chronic renal failure. *Am J Kidney Dis* 29:66–71, 1997
65. Slatopolsky EA, Burke SK, Dillon MA, The RenaGel Study Group: RenaGel, nonabsorbed calcium- and aluminum-free phosphate binder, lowers serum phosphorus and parathyroid hormone. *Kidney Int* 55:299–307, 1999
66. Goldberg DI, Dillon MA, Slatopolsky EA, *et al*: Effect of RenaGel, a non-absorbed, calcium- and aluminum-free phosphate binder, on serum phosphorus, calcium, and intact parathyroid hormone in end-stage renal disease patients. *Nephrol Dial Transplant* 13:2303–2310, 1998
67. Mai ML, Emmett M, Sheikh MS, *et al*: Calcium acetate, an effective phosphorus binder in patients with renal failure. *Kidney Int* 36:690–695, 1989